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ACUTE AND SUBACUTE TOXICITY OF ETHANOLIC EXTRACT OF CYPERUS PAPYRUS IN WISTER ALBINO RATS

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ABSTRACT

Cyperus papyrus (CP) is belong to cyperaceous family. It contains a various secondary metabolites and minerals, and it used traditionally in various situations. As there was no toxicological data found for this plant, acute and sub-acute oral toxicity studies of the ethanolic extracts of (CP) were carried out in Wistar Albino rats, to evaluate its potential toxicity. Acute and sub-acute oral toxicity studies were carried out according to OECD guidelines. Acute exposure of CP extract was studied in rats by oral single dosing of 2000mg/kg BW. Sub-acute oral toxicity was carried out by daily oral administration of CP ethanolic extracts at doses of 175mg/kg, 550mg/kg, 1750mg/kg, to three groups of 6 rats in each, and 1750mg/kg was repeated to the fourth group of 6 rats (as recovery group), for successive 28 day. Control group received distilled water. On day 29, the rats were weighed and sacrificed except the recovery group which sacrificed after extra 14 days without administration of testing compounds. Blood samples were collected for biochemical and hematological analysis and histopathological examination of the vital organs was done. No deaths or signs of acute oral toxicity were recorded. Results obtained after oral sub-acute toxicity showed decrease in RBCs count and HC% which return to the normal range in the recovery group. Biochemical tests showed mild increase in SCr, ALT, AST and ALP that also return to the normal range in the recovery group. histopathological examination doesn't reveal any morphological changes of kidney, liver, lung, spleen, heart, or small intestine. The

results obtained indicate that the LD50 of CP ethanolic extract is greater than 2000 mg/kg and is considered safe and non-toxic at acute exposure.

KEYWORDS: Cyperus Papyrus, acute toxicity, sub-acute toxicity, OECD.

INTRODUCTION

The use of medicinal plants for treatment and management of various diseases and its complications is increasing. Plants contain a variety of phytochemicals that have been proven to be protective by reducing the risk of various ailments and diseases.**(**Ajebli, Khan et al. 2021**).** 80% of the world's population presently uses herbal medicine for some aspect of primary health care according to a survey conducted by World Health Organization(Anjum, Mushtaq et al. 2020).

Cyperus papyrus belongs to the family *cyperaceae* these are monocotyledonous graminoid flowering plants known as sedges, which superficially resemble grasses or rushes.(Kakarla, Allu et al. 2014). It contains small contents of sesquiterpenes relative to monoterpenes obtained by gas chromatography, it also contains phenolic compounds**,** it also contains Na, K, Mg, Fe, I, and proteins which considered as micronutrients (Hassanein, Nazif et al. 2014), Gamal, Hani et al. (2015).*Cyperus papyrus* is used "*traditionally*" in management of painful spasm, eye diseases, ulcers, fever, diarrhea, and inflammations.

Acute and sub-acute toxicity testing was recently carried out according to the OECD guidelines, this test procedure is of principal value in minimizing the number of animals required to estimate the acute oral toxicity of a chemical and in estimating a median lethal dose (LD50%). The median lethal dose allows for comparison with historical data. In addition to the observation of mortality, it allows the observation of signs of toxicity. The latter is useful for classification purposes and in the planning of additional toxicity test (OECD 425, 1998).

In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses (sub-acute toxicity) may be carried out after initial information on toxicity has been obtained by acute toxicity testing. This testing guideline is intended to investigate effects on a very broad variety of potential targets of toxicity. It provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time (OECD 407, 2008). There was no toxicological data

were found for this plant, so this study aims to evaluate the acute and sub-acute oral toxicity of the ethanolic extract of Cyperus Papyrus in rats.

MATERIALS AND METHOD

Plant Material and preparation of plant extract

Fresh aerial parts were harvested from their natural habitat in Khartoum, Sudan in August 2019. Plant identified and a voucher specimen No. (Y-2010-54-MAPTRI-H) was deposited in the herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTRI), National Centre for Research (NCR) Khartoum, Sudan. The freshly harvested leaves were then air dried, 100 g of plant sample was coarsely powdered, extracted with 80 % ethanol according to the method described by (Sukhdev *et al* 2008). Extract was air dried and stored in sealed containers at 4^{o}C until further use.

Experimental Animals

Male and female Wistar albino rats (65 – 120g) obtained from the animal house of MAPTRI, NCR were used for the acute and sub-acute toxicity studies. They were housed in Polypropylene (485*350*200 mm) cages up to a maximum of 6 per cage, in a well-ventilated room with 12 h light/dark cycle, with free access to clean drinking water and food (standard rat feed). They were allowed to acclimatize for one week before experimentation

Phytochemical screening

Phytochemical screening for the active constituents was carried out on ethanolic extract using the methods described by (Martinez & Valencia (1999), Sofowora (1993), Harborne (1984) and Wall et al (1952)) with many few modifications for identification of tannins, triterpenes, alkaloids, flavonoids, saponins, steroids, coumarins, and anthraquinone glycosides.

Acute Toxicity Testing

The acute oral toxicity of the ethanol extract of *CP* was evaluated in Wistar albino rats according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD 425 guideline). Following the adaptation period, the rats were weighed and the dose was calculated in reference to the body weight. The Plant was suspended in a distilled water. For the limit test a single high dose of 2000mg/kg of plant extract. were administered orally (to 5 animals). For the main test a single high dose of 2000mg/kg of plant extract were administered orally (to 5 animals) as test group, whereas the control group received distilled water (10ml/kg) orally. Food was provided to the rats approximately an

hour after treatment and water provided ad libitum. The animals were observed 30min after dosing,1, 2, 4, 24, and 48 hours for short term outcome followed by observation once a day for the next 12 days. All observations were systematically recorded with individual records being maintained for each animal. Animals were weighed on day 1, 7 and 14. food consumption was measured daily, and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were monitored daily during the period.

Sub-acute Toxicity Testing

Sub-acute toxicity of ethanolic extract of CP was evaluated in Wistar albino rats according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD 407 guidelines). rats of both sexes were assigned randomly to five groups (A, B, C, D, E) 6 rats in each. Groups A B C D, received plant extract at doses 1750, 550, 175mg/kg and 1750mg/kg BW (recovery group) respectively. Whereas groups E served as control and received distilled water 10ml/kg BW. The rats were dosed by oral gavage, for 28 days. On day 29, all rats of group A, B, C, and E were sacrificed after an overnight fast, under diethyl ether anesthesia, whereas the remaining 6 rats of groups D (recovery group) were sacrificed in like manner after extra 14 days without administration of testing compounds. Blood was collected for hematological and serum biochemical analysis through the orbital veins. The liver, kidney, lung, spleen, small intestine, and heart were harvested immediately clean of blood using physiological saline then fixed in 10% formalin for histopathological examination.

Histopathology examination

The fixed organs were dehydrated with 100% ethanol solution and embedded in paraffin. They were then processed into 6-8 microns by using microtone and then stained using hematoxylin-eosin (H&E) and observed under light microscope (X40) by histopathologist as earlier described by Gabe (Gabe et al 1968).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism Version 5 software. Data obtained was expressed as Mean ± Standard Error of Mean. The one-way anova (Dunnett's Multiple Comparison Test) was conducted to determine significant differences and p values for significant difference between the mean of control and test groups was considered at $p < 0.05$.

RESULTS

Phytochemical screening

Preliminary phytochemical analysis of the extract revealed the presence of saponin, flavonoids, tannin, and steroids with different percentage.

Acute Toxicity Testing

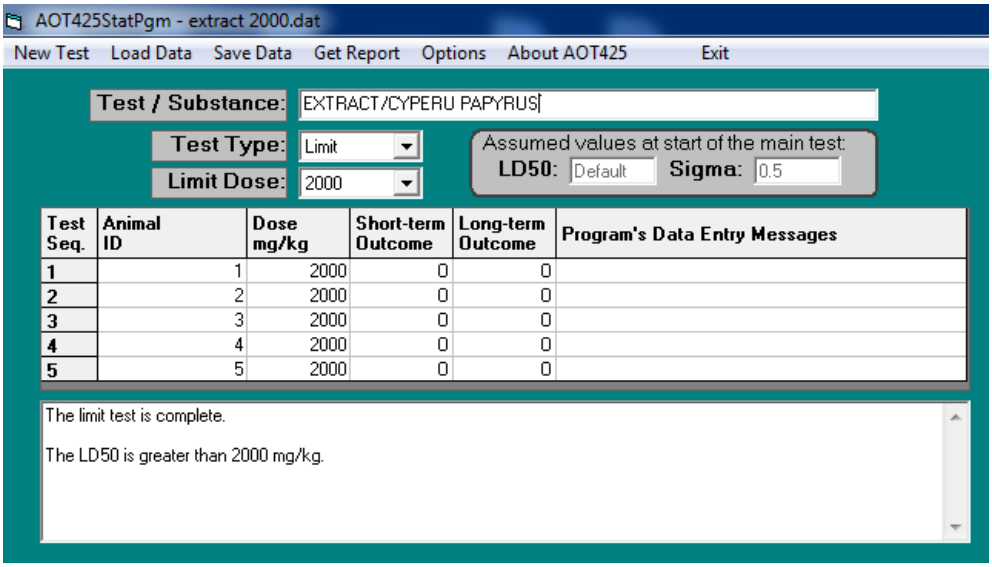

Figure 1: acute limit test of *CP* extract at dose of 2000mg/kg-1BW.

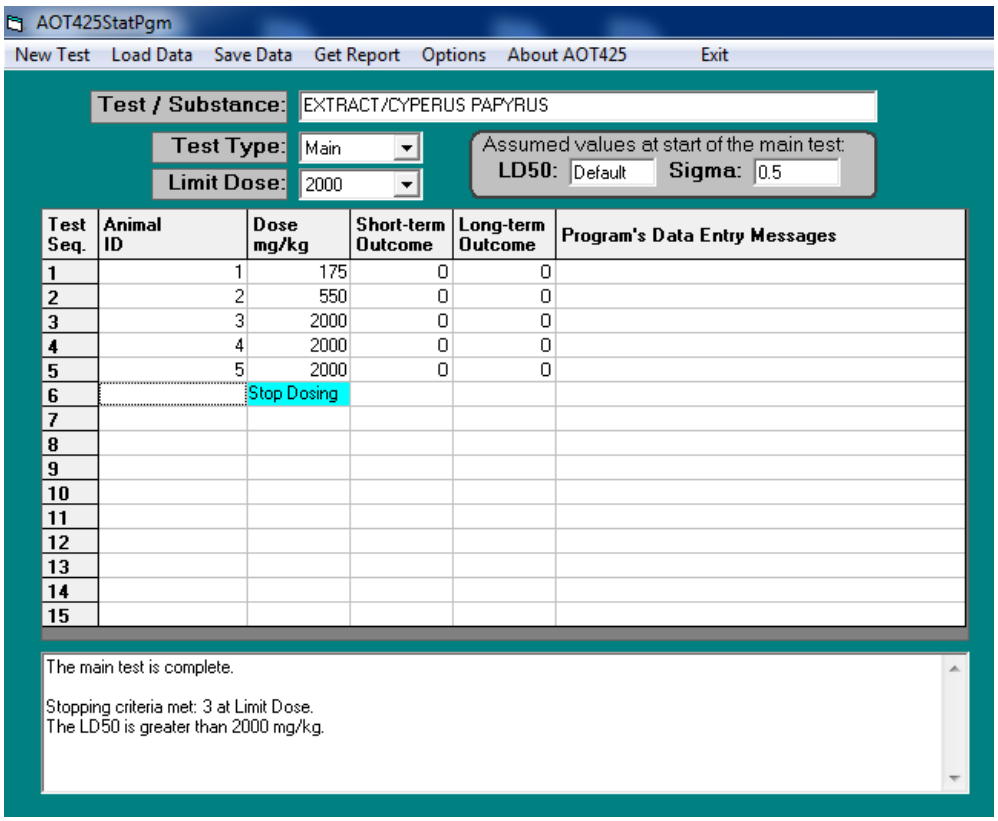

Figure 2: acute main test of CP extract at dose of 2000mg/kg-1BW.

Table 1: Lethality and behavioral analysis during acute toxicity test of CP ethanolic extract.

Observation	STO Day 1										STO Day 2		LTO			
Time	30 min		1hr		2hr		4hr		24hr		48hr		Day7		Day 14	
Group	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Food consume	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin and fur change	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes change	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhoea	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Respiraton	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Heart rat	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Blood pressure	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremors	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mortality time											-		-		-	

N; normal, STO; short term outcome, LTO; long term outcome, C: Control group, E: Ethanolic extract of CP group.

Table 2: Histopathology (Necropsy) and macroscopical observations after acute toxicity test of CP ethanolic extract.

Group	C	E
Heart	N	N
Lung	N	N
Liver	N	N
Spleen	N	N
Kidney	N	N
GIT	N	N

N; normal, C: Control group, E: Ethanolic extract of CP group.

No mortality was recorded in both male and female rats administered the ethanol extract of *CP* at a dose of 2000 mg/kg-BW as shown in Figure 1.

Table 3: Change in body Weight of animals during acute toxicity testing (2000mg/kg) of CP extract.

Parameters	Doses	Mean± SEM	Significance
Body weight initial week1	Control	4.40± 0.75	0.0637
	extract	**7.80 ± 2.63**	
Body weight week2	**Control**	**7.00±1.05**	**0.6078**
	extract	**6.40± 2.50**	

Results represented as mean and SEM: standard error of mean.

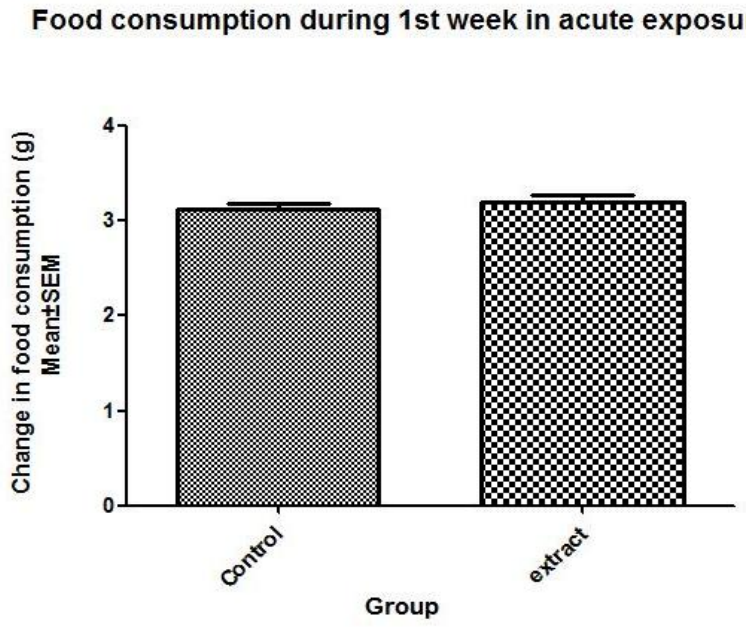

Figure 3: Represents the change in food consumption during 1st week at acute exposure of CP ethanolic extract.

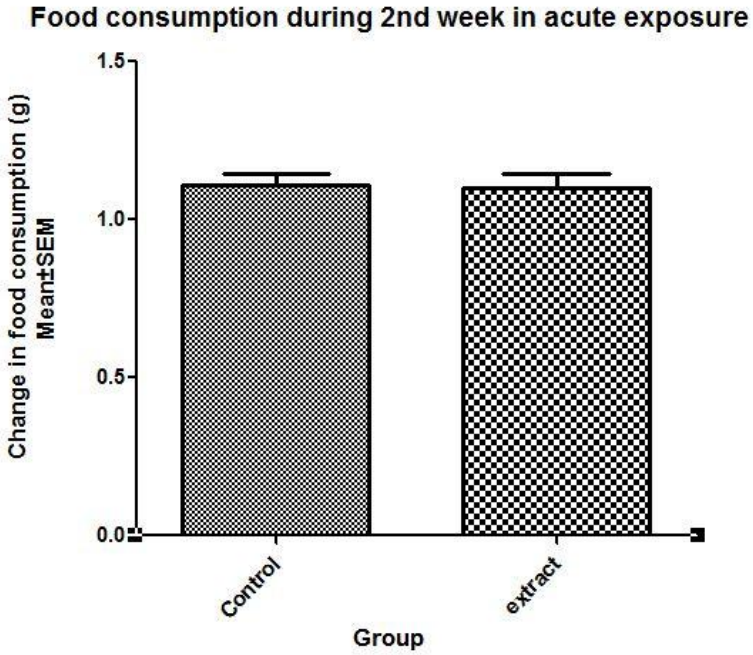

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Figure 4: Represents the change in food consumption during 2nd week at acute exposure of CP ethanolic extract.

Subacute toxicity

Weight changes during subacute toxicity test of CP extract

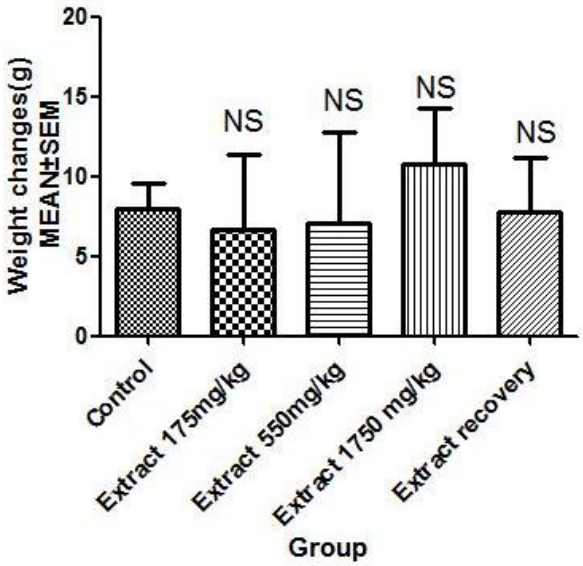

Figure 5: Represents the change in animal body weight during subacute exposure of CP ethanolic extract.

Table 4: Effect of *CP* extract on some hematological parameters in rats.

Group / Parameters	Mean ± SEM (P value)				
	Control	**Low dose 175mg/kg**	**Intermediate dose 550mg/kg**	**High dose 1750mg/kg**	**Recovery 1750mg/kg**
TWBCs Cells/µL	8067±1189	6533±421.6 **(NS)**	9100±1558 **(NS)**	7300±1024 **(NS)**	9250±396.4 **(NS)**
RBCs Cells/ µL	$8.417*10^6$ ± 178151	$7.753*10^6$ ± 90247 **(**)**	$7.658*10^6$± 113942 **(**)**	$7.703*10^6$± 88305 **(**)**	$8.535*10^6$± 189697 **(NS)**
HGB g/dL	14.72±0.52	14.05±0.18 **(NS)**	13.45±0.24 **(NS)**	13.17±0.24 **(*)**	15.08±0.41 **(NS)**
HCT %	51.4±1.16	46.6±0.44 **(***)**	44.9±0.67 **(***)**	44.25±0.42 **(***)**	53.8±0.93 **(NS)**
MCV	61.33±2.04	59.65±0.26 **(NS)**	58.65±0.65 **(NS)**	57.47±0.13 **(NS)**	63.07±0.89 **(NS)**
MCH	17.08±0.08	17.95±0.09 **(*)**	17.55±0.34 **(NS)**	17.1±0.25 **(NS)**	17.67±0.15 **(NS)**
MCHC	28.72±1.27	30.12±0.12 **(NS)**	29.97±0.33 **(NS)**	29.75±0.41 **(NS)**	28.07±0.45 **(NS)**
PLT	$1.013*10^6$± 63877	735500 ± 56762 **(*)**	939833 ± 103630 **(NS)**	762333 ± 48742 **(NS)**	962333 ± 73810 **(NS)**
LYM%	74.3±2.71	54.17±4.53 **(**)**	61.05±4.33 **(NS)**	60.62±2.5 **(NS)**	80.58±6.19 **(NS)**

LYM	6083±994.5	3633±449.2 **(NS)**	5683±1250 **(NS)**	4517±664.5 **(NS)**	7333±304 **(NS)**
RDW-SD	36.28±2.04	35.45±0.84 **(NS)**	33.27±1.4 **(NS)**	31.58±0.64 **(*)**	36.77±0.47 **(NS)**
PDW	9.05±0.22	9.4±0.16 **(NS)**	9.06±0.25 **(NS)**	9.2±0.13 **(NS)**	9.1±0.2 **(NS)**
MPV	7.36±0.15	7.8±0.05 **(NS)**	7.63±0.13 **(NS)**	7.58±0.06 **(NS)**	7.56±0.16 **(NS)**
P-LCR%	10.07±1.08	11.17±0.29 **(NS)**	10.03±0.91 **(NS)**	10.32±0.38 **(NS)**	9.86±1.23 **(NS)**
RDW-CV%	16.82±0.37	17.53±0.53 **(NS)**	15.78±0.86 **(NS)**	14.77±0.46 **(*)**	16.58±0.25 **(NS)**

SEM: standard error of mean, (*) p value ≤0.05, (**) P value ≤0.01, (***) P value ≤0.001, (NS) non-significant difference i.e. P value ≥0.05, (WBC) White blood cell, (RBC) red blood cell, (PLT) platelet counts, (HGB) hemoglobin concentration, (HCT) hematocrit, (MCV) mean corpuscular volume, (MCH) mean corpuscular hemoglobin, (MCHC) mean corpuscular hemoglobin concentration, (LYM) lymphocyte count, (RDW) red cell distribution width, (PDW) platelet distribution width, (MPV) mean platelet volume, (P-LCR) platelet large cell ratio.

Table 5: Effect of *CP* extract on some biochemical parameters in rats.

Group / Parameters	Mean ± SEM (P value)				
	Control	**Low dose 175mg/kg**	**Intermediate dose 550mg/kg**	**High dose 1750mg/kg**	**Recovery 1750mg/kg**
SCr	0.468±0.13	0.73±0.018 **(NS)**	1.21±0.25 **(*)**	1.297±0.205 **(**)**	0.886±0.116 **(NS)**
BUN	31.83±7.53	39.33±1.2 **(NS)**	32.33±9.53 **(NS)**	30.17±5.94 **(NS)**	74.5±12.15 **(**)**
Total Bilirubin	0.042±0.01	0.69±0.04 **(**)**	0.37±0.25 **(NS)**	0.438±0.14 **(NS)**	0.04±0.0069 **(NS)**
Direct Bilirubin	0.024±0.007	0.116±0.07 **(NS)**	0.038±0.019 **(NS)**	0.041±0.012 **(NS)**	0.003±0.001 **(NS)**
AST	109.5±21.98	166.8±7.66 **(*)**	139.8±11.68 **(NS)**	143.8±8.28 **(NS)**	146.5±20.2 **(NS)**
ALT	14.83±3.04	57.83±10.5 **(**)**	18.33±3.68 **(NS)**	21±5.27 **(NS)**	40.5±10.88 **(NS)**
ALP	85.83±15.91	195.5±3.19 **(*)**	124±34.2 **(NS)**	126.3±37.06 **(NS)**	138.5±26.9 **(NS)**
Total Protein	4.77±0.739	6.56±0.033 **(NS)**	3.71±1.12 **(NS)**	3.04±1.17 **(NS)**	5.9±1.0 **(NS)**
Albumin	2.45±0.287	3.13±0.07 **(NS)**	1.86±0.507 **(NS)**	1.923±0.43 **(NS)**	3.45±0.77 **(NS)**

SEM: standard error of mean, (*) p value ≤0.05, (**) P value ≤0.01, (NS) non-significant difference i.e. P value ≥0.05, (SCr) serum creatinine, (BUN) blood urea nitrogen, (AST)aspartate transaminase, (ALT)alanine transaminase, (ALP)alkaline phosphatase.

Histopathology Findings

No any morphological differences observed between extract groups and control group in kidney, liver, spleen, lung, heart, or small intestine according to figures below.

Effect of ethanolic extract of CP on heart histopathology during sub-acute exposure

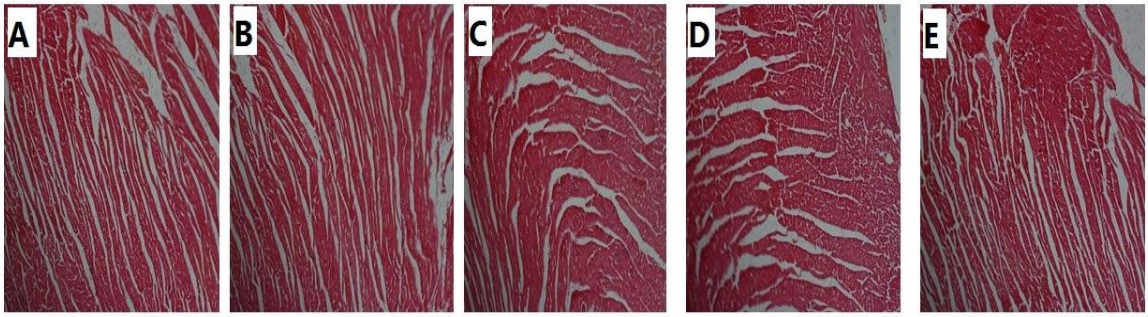

Figure 6: The histopathology changes of heart after sub-acute exposure, represented as (A) control group, (B) 175mg/kg CP extract, (C) 550mg/kg CP extract, (D) 1750mg/kg CP extract, and (E) recovery group 1750mg/kg CP extract using light microscope (H & E × 40).

Effect of ethanolic extract of CP on lung histopathology during sub-acute exposure

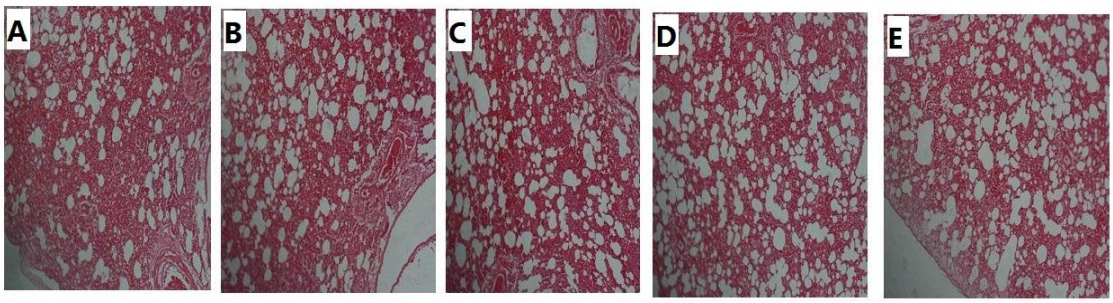

Figure 7: The histopathology changes of lung after sub-acute exposure, represented as (A) control group, (B) 175mg/kg CP extract, (C) 550mg/kg CP extract, (D) 1750mg/kg CP extract, and (E) recovery group 1750mg/kg CP extract using light microscope (H & E × 40).

Effect of ethanolic extract of CP on kidney histopathology during sub-acute exposure

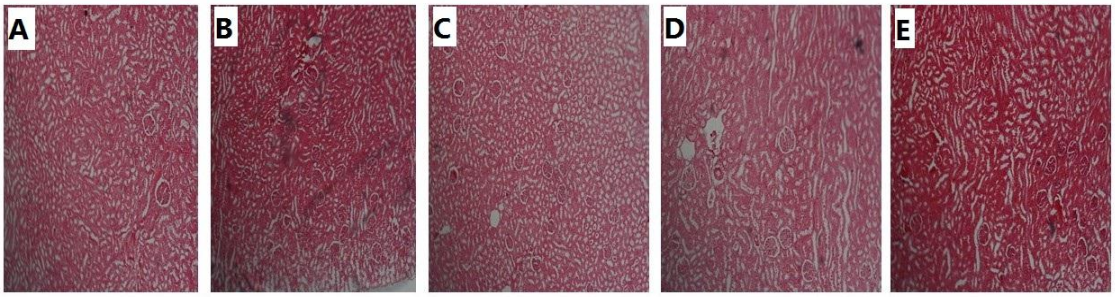

Figure 8: The histopathology changes of kidney after sub-acute exposure, represented as (A) control group, (B) 175mg/kg CP extract, (C) 550mg/kg CP extract, (D) 1750mg/kg CP extract, and (E) recovery group 1750mg/kg CP extract using light microscope (H & E × 40).

Effect of ethanolic extract of CP on liver histopathology during sub-acute exposure

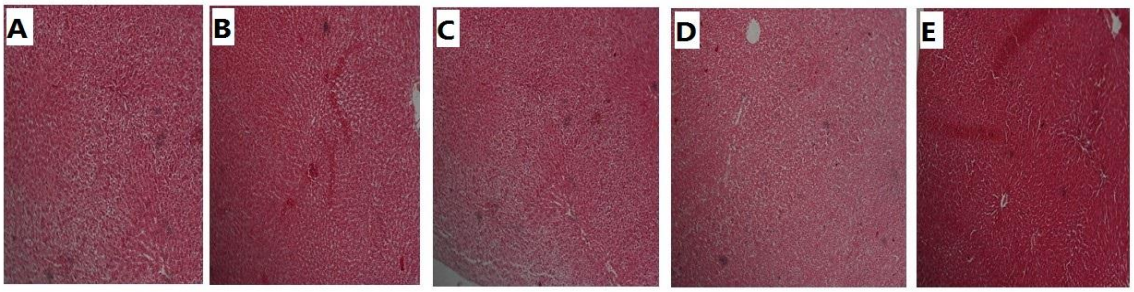

Figure 9: The histopathology changes of liver after sub-acute exposure, represented as (A) control group, (B) 175mg/kg CP extract, (C) 550mg/kg CP extract, (D) 1750mg/kg CP extract, and (E) recovery group 1750mg/kg CP extract using light microscope (H & E × 40).

Effect of ethanolic extract of CP on spleen histopathology during sub-acute exposure

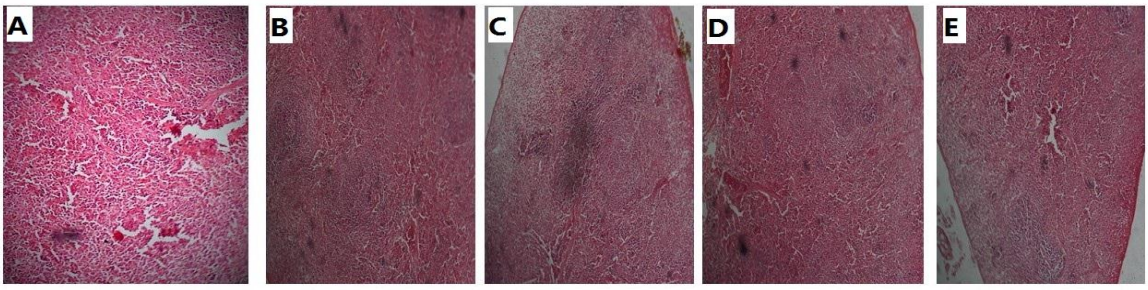

Figure 10: The histopathology changes of spleen after sub-acute exposure, represented as (A) control group, (B) 175mg/kg CP extract, (C) 550mg/kg CP extract, (D) 1750mg/kg CP extract, and (E) recovery group 1750mg/kg CP extract using light microscope (H & E × 40).

Effect of ethanolic extract of CP on small intestine histopathology during sub-acute exposure

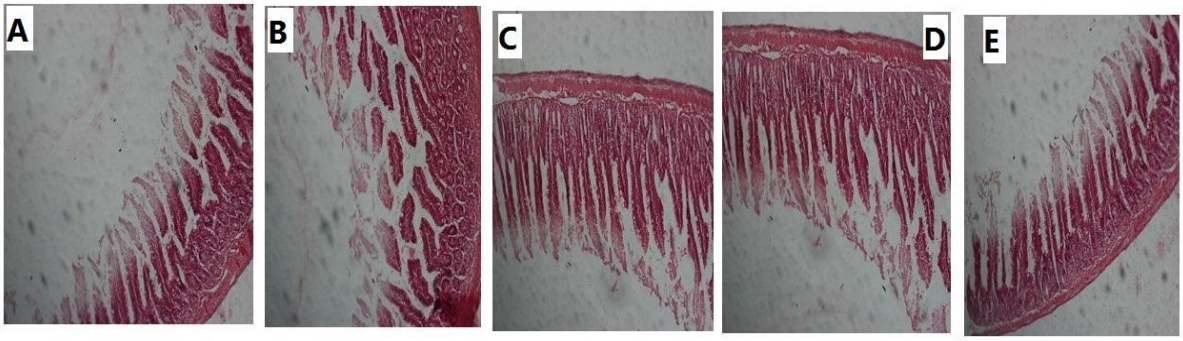

Figure 11: The histopathology changes of small intestine after sub-acute exposure, represented as (A) control group, (B) 175mg/kg CP extract, (C) 550mg/kg CP extract, (D) 1750mg/kg CP extract, and (E) recovery group 1750mg/kg CP extract using light microscope (H & E × 40).

DISCUSSION

The ethanolic extract of CP contains flavonoid, steroids, saponin, and tannins to which attributed the plant characters and properties.

In acute toxicity, rats treated with ethanol_extracts of CP at dose of 2000mg/kg BW, there was no mortality or any signs of toxicity or side effects recorded. The test animals did not show any significant changes in behavioral pattern such as trembling, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, hair loss, sleep, lethargy, restlessness, or in physical appearance such as eye color, mucous membrane, salivation, skin/fur effects, coma, body weight(Table 1&2), injury, change in heart rate or blood pressure, when compared to the control throughout the 14 days.

No histopathology changes (Necropsy) of heart, lung, liver, spleen, kidney or GIT of tested animals observed when compared to the control at the end of 14 days. The food consumption, body and organs weights of experimental animals did not show any significant changes, when compared to the control group. Compounds with LD50 values lower than 2000mg/Kg -BW are generally considered to be relatively safe, whereas values above this are non-classified. Thus, the ethanol extracts of *CP* can be considered to be nontoxic at acute administration level, since the extract was well tolerated and there was no adverse effect was observed.

Analysis of blood parameters in animal studies is relevant to evaluate the risk of alterations of the hematopoietic system in toxicity studies, for necessary application to humans. Regarding sub acute treatment, hematological parameters analyzed included the complete blood count of experimental and control group animals. In this study, after 28 days of administration of the extract, there was very highly significant decrease in hematocrit (HCT) ($p<0.001$), and in red blood cell count (RBC) ($p<0.01$), at all doses level of treatment. The extract at a dose 1750mg/kg. showed a significant ($p<0.05$) decrease in hemoglobin (HGB) level, and significant decrease (($p<0.05$) red cell distribution width (RDW). The plant extract at a dose 175mg/kg, showed a significant decrease ($p<0.01$) in lymphocyte percent (LYM%) and significant increase ($p<0.05$) in mean corpuscular hemoglobin (MCH) and significant decrease ($p<0.05$) in Platelet count (PLT) compared to the control, all these abnormalities were return to the normal range after 14 day in the recovery group. The rest of the parameters such as TWBCs, MCV, MCHC, LYM, PDW, MPV, and P-LCR% did not change significantly compared to the control group as shown in table (4).

The effect of the extract of *CP* on biochemical parameters as shown in table (5), a highly significant increase ($p<0.01$) in serum creatinine level at a dose 1750mg/kg, and significant increase ($p<0.05$) in serum creatinine level at a dose 550mg/kg were observed. A highly significant increase ($p<0.01$) in total bilirubin level,, and highly significant increase ($p<0.01$) in ALT level at dose 175mg/kg. Both AST and ALP levels are significantly increase ($p<0.05$) at dose 175mg/kg compared to the control group, and all these abnormalities were return to the normal range after 14 days in the recovery group, whereas BUN was highly significant ($p<0.01$) increased in recovery group only. The rest of the parameters including direct bilirubin, total protein, and albumin did not change significantly compared to the control group.

Effect of *CP* extract on some histopathological parameters in rats**:** Revealed no tubular necrosis, no change in glomeruli, and no cellular infiltrate in interstitial of **kidney** were observed in all rat groups that received CP extract when compared to the control group. There was no necrosis in the **liver**, no inflammatory cellular infiltrate, and no stasis in bile cuniculi of kidney in all rat groups that received CP extract when compared to the control group. There was no congestion in red pulp of **the spleen**, no paucity of white pulp, and no increase in hemosiderin macrophage level were observed in all rat groups that received CP extract when compared to the control group. No necrosis, no inflammatory cells in alveoli, no

mucus or edema in alveoli, and no mucus in bronchioles were observed in the **lung** of all rat groups that received CP extract when compared with the control group. No necrosis in heart, and no inflammatory cellular infiltrate were seen in all rat groups that received CP extract when compared with the control group. In the small intestine normal villi was observed, no inflammatory cells in lumen of propria in all rat groups that received CP extract when compared with the control group.

CONCLUSION

The oral LD50 of ethanolic extract of C P has been shown to be greater than 2000 mg/kg and is generally considered safe at acute exposure without any other abnormalities. Whilst upon sub-acute exposure the CP ethanolic extract did show some hematological and biochemical abnormalities, which return to the normal range with recovery group and without any histopathological changes. There was an increase in BUN level with recovery group and this may be due to delayed toxicity, a further sub-chronic study would be required to approve this abnormality.

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